Amendments to the Specification:

On page 1, after the title, please insert the following:

This is the national phase of PCT/IB2004/003726 filed November 4, 2004, which claims priority to U.S. 60/517,584, filed November 5, 2003 and GB 0406296.4 filed March 19, 2004, the entire contents of which are incorporated.

FIELD OF THE INVENTION

On page 1, on line 7, before the sentence beginning with "Plastids are membrane-bound organelles. . ." please insert the following:

BACKGROUND

On page 1 line 26, please insert the following text:

SUMMARY OF THE INVENTION

The present invention provides a method of producing a recombinant polypeptide, comprising transferring a recombinant polypeptide which is glycosylated in the ER of a plant cell to a plastid in the plant cell. The invention also provides a method of producing a recombinant polypeptide comprising expressing in a plant cell a nucleic acid encoding a fusion polypeptide which comprises an ER signal sequence, one or more ER-plastid targeting sequences and a heterologous recombinant polypeptide. The plant ER signal sequence may be from an ER processed plastid polypeptide. The ER-plastid targeting sequences may comprise at least 10 contiguous amino acids from an ER-processed plastid polypeptide. In some embodiments the at least 10 contiguous amino acids may comprise two or more contiguous basic residues.

In some embodiments, the ER-plastid targeting sequences are comprised within an ER-processed plastid polypeptide.

In preferred embodiments the ER-processed plastid polypeptide has a sequence listed in Table 1. In one embodiment the ER-processed plastid-localised polypeptide is a CAH1 polypeptide.

In yet another embodiment, there is provided a method of producing a recombinant polypeptide comprising expressing in a plant cell a nucleic acid encoding a fusion polypeptide which comprises an ER signal sequence, one or more ER-plastid targeting sequences and a heterologous recombinant polypeptide, and further comprises cleaving the expressed fusion polypeptide to generate the recombinant polypeptide. The expressed fusion polypeptide may comprises one or more cleavable linker sequences, and the heterologous polypeptide is generated by cleavage of the one or more linker sequences. The one or more linker sequences may be cleaved within the plastid by a heterologous endoprotease to generate the recombinant polypeptide. The method according to claim 10 wherein said one or more linker sequences are cleaved within said plastid by an endogenous plastid endoprotease to generate said recombinant polypeptide.

In another embodiment, there is provided a method of producing a recombinant polypeptide comprising expressing in a plant cell a nucleic acid encoding a fusion polypeptide which comprises an ER signal sequence, one or more ER-plastid targeting sequences and a heterologous recombinant polypeptide, and further comprises isolating and/or purifying the recombinant polypeptide from a plastid of the cell. The isolating and/or purifying of the expressed fusion polypeptide from a plastid of the cell may be performed prior to cleavage to generate the recombinant polypeptide.

In some embodiments, the recombinant polypeptide comprises one or more glycosylation sites. Further, the the glycosylation of the expressed recombinant polypeptide may be determined.

In some embodiments of the invention, the plastid is preferably a chloroplast.

The present invention also provides a nucleic acid construct comprising a nucleotide sequence that encodes an ER signal sequence, and one or more ER-plastid targeting sequences; one or more restriction endonuclease sites for insertion of a nucleotide coding sequence capable of expressing a recombinant polypeptide fused to said ER signal and ER-plastid targeting sequences, and; a heterologous regulatory sequence operably linked to the nucleotide sequence.

The nucleic acid construct may also comprise a heterologous nucleotide coding sequence capable of expressing a recombinant polypeptide fused to said ER signal and ER-plastid targeting sequences, said coding sequence being inserted in the one or more restriction endonuclease sites.

In some embodiments, the nucleotide sequence further encodes one or more cleavable linker sequences, said recombinant polypeptide being generated by cleavage of said one or more linker sequences.

In some embodiments, the ER signal sequence is from an ER-processed plastid polypeptide and in some embodiments the one or more ER-plastid targeting sequences comprise at least 10 contiguous amino acids from an ER-processed plastid polypeptide. In some embodiments, the one or more ER-plastid targeting sequences comprise two or more contiguous basic residues and in some embodiments, the ER signal sequence and one or more ER-plastid targeting sequences are comprised within an ER-processed plastid polypeptide sequence. The ER-processed plastid polypeptide sequence is a sequence listed in Table 1 and in some embodiments, the ER-processed plastid polypeptide sequence is a CAH1 polypeptide. In some embodiments, the plastid is a chloroplast.

The present invention also provides a nucleic acid vector suitable for transformation of a plant cell and comprising nucleic acid constructs of the present invention.

The present invention also provides a host cell comprising a nucleic acid construct of the present invention. The host cell may have the nucleic acid construct or vector within its genome. The host cell may be a plant cell. The plant cell preferably comprises a nucleic acid encoding one or more mammalian glycosyltransferases. The plant cell may be deficient in one or more plant specific glycosyltransferases.

The present invention provides a plant cell as described above, which is comprised in a plant, a plant part or a plant propagule, or extract or derivative of a plant.

The present invention also provides a method of producing host cells of the present invention, the method comprising incorporating said nucleic acid construct or vector into the cell by means of transformation. In some embodiments, the nucleic acid is combined with the cell genome nucleic acid such that it is stably incorporated therein. In some embodiments, a plant is regenerated from one or more transformed cells. In some embodiments, a method of producing host cells further comprises sexually or asexually propagating or growing off-spring or a descendant of the plant regenerated from said plant cell.

The present invention also provides a plant comprising a host cell of the present invention.

The present invention also provides a method of producing a plant comprising incorporating a nucleic acid construct of the present invention into a plant cell and regenerating a plant from said plant cell.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the deduced amino acid sequence of CAH1. The arrow indicates the predicted signal peptide cleavage site. Underlined triplets indicate possible *N*-glycosylation sites.

Figure 2 shows the nucleotide sequence of Arabidopsis CAH1.

Figure 3 shows the distribution of the antimycine A resistant NADH cytochrome c reductase activity and CAH1 isoforms following fractionation of the total microsome fraction from both control and BFA-treated cells over a sucrose density gradient.

Figure 4 shows the structure of the GFP-tagged and truncated forms of the Arabidopsis CAH1 protein used to localize the domain required for plastid localization. (1-40)CAH1, GFP-fusion containing the signal peptide for the ER (first 40 amino acids). (1-103)CAH1, GFP-fusion containing the first 103 amino acids of the CAH1. (1-40)CAH1-GFP-(224-284)CAH1, GFP-fusion containing the signal peptide for the ER (first 40 amino acids) plus the last 61 amino acid residues of the CAH1.

DETAILED DESCRIPTION OF THE INVENTION

On page 19, line 8, before the text "Experimental Materials and Methods" please insert the following:

EXAMPLES

On page 22, line 4, please delete the paragraph and replace with the following new paragraph:

2D-electrophoresis

Stroma samples containing 300-400 µg of protein were precipitated with 0.15 % (v/v) deoxycholic acid and 72 % (v/v) TCA as described[[^{33}]] (Goulas, E., *et al. Annals Botany* 88, 789-795 (2001)) and solubilized in 2D rehydration solution, containing 8 M urea, 2 % (w/v) CHAPS, and 0.002 % (w/v) bromophenol blue. The solubilized samples were loaded onto

linear immobilized pH gradient gels (IPG) covering the pH ranges from 4-7 and 3-10 (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The samples were applied by in-gel-rehydration and isolelectrically focused using an IPGphor system (Amersham Pharmacia Biotech AB). After focusing, strips were equilibrated twice, for 15 min each time, in equilibration buffer (50 mM Tris-HCl (pH 8.8), 6 M urea, 30 % (v/v) glycerol, 0.002 % (v/v) bromophenol blue, and 2 % (w/v) SDS), containing 1 % (w/v) DTT in the first equilibration, and 2.5 % (w/v) iodoacetamide in the second. After the equilibration steps, the strips were loaded onto 10 % SDS-PAGE gels, and electrophoretically separated at constant current. After 2D protein separation, stroma proteins were detected using a silver-staining method as described (Blum, H. *et al.*, *Electrophoresis.* 8, 93-99 (1987)), or were electrotransferred onto nitrocellulose membrane. The membranes were then incubated with antibodies raised against CAH1, β(1,2)-xylose, and α(1,3)-fucose epitopes.

On page 24, line 17, please delete the paragraph and replace with the following new paragraph:

Construction of GFP reporter plasmids for transient expression in Arabidopsis and tobacco cells

The GFP reporter plasmid 35Ω-sGFP(S65T) and the plasmid containing the transit peptide (TP) sequence from RBCS fused to GFP (35Ω-TP-sGFP(S65T)) have been previously described. [[39]] (Chiu, W-L., *et al. Current Biol.* 6, 325-330 (1996)). The plasmids for expression of truncated *Arabidopsis* CAH1 protein fused to GFP were constructed as follows: The CaMV35S-CAH1-sGFP(S65T) corresponding to the coding region of *Arabidopsis* CAH1 was PCR-amplified using the two flanking primers for-*SalI* (TAAAAGTCGACATGAAGATTATGATGATGA) and rev1-*NcoI* (AAAACCCATGGAATTGGGTTTTTTCTTTTT) and the PCR product was cloned into the *SalI-NcoI* digested GFP reporter plasmid CaMV35S-sGFP(S65T). The protocol was similar for the other constructions. The CaMV35S-(1-40)CAH1-sGFP(S65T) corresponding to CAH1 containing the first 40 amino acids was PCR amplified using the two flanking primers for-*SalI* and rev2-*NcoI* (GTGTCCCATGGGGTTTGGTCCATTTTTGCC). The CaMV35S-(1-103)CAH1-sGFP(S65T) corresponding to CAH1 containing the first 103 amino acids was PCR amplified using the two flanking primers for-*SalI* and rev3-*NcoI* (TATCACCATGGCTGCTCCCTCCCCGAAGA). The CaMV35S-(1-40)CAH1-

sGFP(S65T)-(224-284)CAH1 corresponding to CAH1 containing the first 40 and last 61 amino acids was PCR amplified using the two flanking primers for-*SalI* and rev2-*NcoI* and the two flanking primers for-*BsrGI* (TTCTTTGTACATCCTTGGCAAGGTGAGGTC) and rev-*BsrGI* (GACAATGTACAACTATTTTAATTGGGTTTT). The CaMV35S-CAH1-sGFP(S65T)-KDEL corresponding to the coding region of Arabidopsis CAH1 fused to a KDEL-tagged GFP was PCR amplified using the two flanking primers for-SalI and rev2-BsrGI:

ACAGTGTACACTAATGGTGATGGTGATGGTGATTGGGTTTTTTCTTTTTGTTACC.

The plasmids were sequenced to check that the orientation and sequences of the inserted fragments were correct. The plasmids used for tissue bombardment were prepared using the QIAfilter [[plamid]] plasmid midi kit (Qiagen Laboratories).